BASIC RESEARCH STUDY

From the Southern Association for Vascular Surgery (William J. von Liebig Award winner)

Inflammation and intimal hyperplasia associated with experimental pulmonary embolism

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Objective: **We tested the hypothesis that a venous thromboembolism to the pulmonary arterial system (pulmonary embolism [PE]) would cause an inflammatory response within the pulmonary arterial (PA) wall marked by elevated cytokines and chemokines and an influx of inflammatory cells.**

Methods: **Experimental PE was induced in 70 rats and confirmed with angiography and O_2 saturation depression, and an additional 70 rats underwent sham operations. PA and lung tissue were removed at 3 hours and at 1, 2, 4, 6, 8, and 14 days (n = 10 per time point), were analyzed for proinflammatory cytokines and chemokines, and underwent histologic analysis. Data were analyzed with analysis of variance and the unpaired Student *t* test.**

Results: **Average gross PE resolution was 40% at 2 days, 90% at 4 days, and 100% at 6 days. Only monocyte chemoattractant protein–1 levels were greater in affected PAs compared with sham PAs at 3 hours, 1 day, and 2 days (137 ± 13 pg/mg protein, 285 ± 40 pg/mg protein, and 249 ± 36 pg/mg protein versus 101 ± 6 pg/mg protein, 150 ± 36 pg/mg protein, and 92 ± 3 pg/mg protein; $P < .01$ for all). Keratinocyte-derived chemokine, tissue necrosis factor, interleukin-10, nitric oxide, P-selectin, and E-selectin levels were not elevated. Neutrophils infiltrated the PA wall beginning at 3 hours, peaked at 2 days (69.4 ± 21.7 per five high-power fields; $P < .01$), and returned to baseline by 8 days after PE. Macrophages peaked at 1 day after PE (29.3 ± 6.9; $P < .01$) and returned to baseline by 4 days after PE. PE also was associated with a significantly increased intima to media ratio ($P < .05$), apparent at 4 days after PE and persisting through 14 days.**

Conclusion: **PE is associated with an early influx of polymorphonuclears and macrophages and monocyte chemoattractant protein–1 elevation within the PA wall. These are temporally associated with thrombus resolution and intimal hyperplasia. These factors may mediate these two processes after PE. This offers targets for further study with the hopes of minimizing the pathophysiologic response to PE. (J Vasc Surg 2002;36:581-8.)**

Pulmonary embolism (PE) is a major health concern with an estimated incidence rate of 69 cases per 100,000 and a prevalence rate of 3.5 cases per 1000 hospital admissions.[1,2] The acute sequelae of a PE can vary from no symptomatology to sudden death. These outcomes and subsequent resolution of pulmonary defects are influenced by initial defect size, prior cardiopulmonary disease, and patient gender.[3] Resolution of pulmonary thromboemboli occurs with endogenous thrombolysis, fragmentation, or organization and recanalization. The latter process has been associated with the development of intraluminal fibrous bands and intimal fibrosis and may contribute to the development of PE-associated pulmonary hypertension.[4,5]

The morphology of a thromboembolus is similar to that of a nondetached thrombus.[6] Extensive research has begun to document the process of thrombus resolution, organization, and recanalization in deep venous thrombosis (DVT). Thrombus development is associated with vein wall inflammation marked by an early extravasation of leukocytes and elevation in proinflammatory mediators and selectins.[7-11] Further thrombus organization involves neovascularization, which can be augmented by interleukin-8.[12] Although the balance between plasminogen activators and type I plasminogen activator inhibitors has been shown to perhaps play a key role in the process of PE resolution, the cellular and chemokine response in the PA wall or parenchyma to an acute PE has not been evaluated.[13] It is unknown, however, whether the process is the same in a

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Table I. Morphometric analysis (cells per five high-power fields) of inflammatory cells in PA wall indexed to time from PE

	Baseline	*3 Hours*	*Day 1*	*Day 2*
PE				
Affected PA (n = 6)				
Total leukocytes	0.4 ± 0.4	15.4 ± 4.4*†	78.4 ± 20.3*†‡	96.0 ± 29.9*†‡
Neutrophils	0	9.6 ± 2.8*†	48.3 ± 17.8*†	69.4 ± 21.7*†‡
Macrophages	0.4 ± 0.4	5.8 ± 3.0	29.3 ± 6.9*†‡	26.6 ± 10.9‡
Lymphocytes	0	0	0.8 ± 0.6	0
Contralateral PA (n = 6)				
Total leukocytes	1.4 ± 0.6	3.3 ± 1.9	2.6 ± 1.1	5.0 ± 2.0
Neutrophils	1.0 ± 0.6	1.3 ± 1.3	0.8 ± 0.6	3.5 ± 1.3
Macrophages	0.4 ± 0.4	1.5 ± 0.6	1.8 ± 1.1	1.5 ± 0.9
Lymphocytes	0	0	0	0
Sham PA (n = 6)				
Total leukocytes	0.8 ± 0.4	0.4 ± 0.4	0.3 ± 0.3	1.0 ± 0.6
Neutrophils	0.4 ± 0.2	0	0.25 ± 0.25	0.4 ± 0.4
Macrophages	0	0	0	0
Lymphocytes	0.4 ± 0.4	0.4 ± 0.2	0	0.6 ± 0.4

*$P < .01$ compared with sham PA.
†$P < .01$ compared with contralateral PA.
‡$P < .01$ compared with baseline.
ANOVA, Analysis of variance; *NS*, not significant.

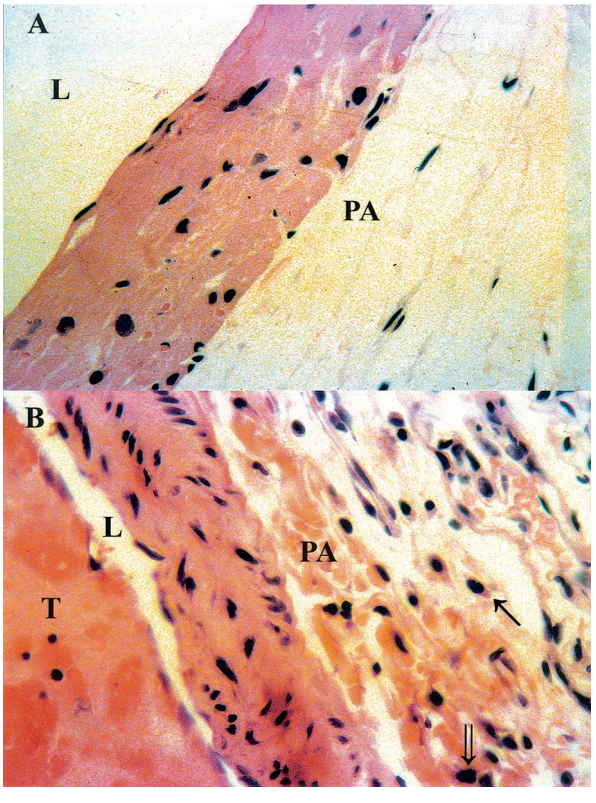

Fig 1. Photomicrographs show: **A,** sham animal (2 days after PE) with few PA wall inflammatory cells and similar histologic appearance to baseline; and **B,** experimental animal 2 days after PE with increased neutrophil and macrophage cellular infiltrate into PA wall and in surrounding lung parenchymal tissue. Hematoxylin and eosin; original magnification, 60×.
T, Thrombus; *L,* lumen; *single arrow,* neutrophil; *double arrow,* macrophage.

thrombus that develops in a vein (DVT) or in a thrombus that develops in a vein and then lodges somewhere else, such as the pulmonary circulation (PE).

The aim of this study was to test the hypothesis that the pulmonary arterial (PA) wall would have an inflammatory response to a PE associated with an elevation of inflammatory cells and proinflammatory cytokines and chemokines. In addition, we tested the hypothesis that PE is associated with parenchymal inflammation and fibrosis.

MATERIALS AND METHODS

Animal model. Seventy Sprague-Dawley rats, weighing 300 to 350 g, were anesthetized with an inhalation mixture of isoflurane (1% to 2%) and oxygen (100%) and had inferior vena caval (IVC) thrombosis induced as previously described.[7-12,14-17] Forty-eight hours later, the thrombus within the IVC was massaged free and embolized proximally. Through a vertical neck incision, the right internal jugular vein was exposed and cannulated with polyethylene tubing (PE-50, Becton Dickinson, Parsippany, NJ). With fluoroscopic guidance (9400 X-ray Imaging System, OEC-Diasonics, Salt Lake City, Utah), the tubing was advanced into the right heart and a pulmonary angiogram (Visipaque 320, Nycomed, Princeton, NJ; diluted 1:1 with normal saline solution) was performed to verify and localize the PE. A maximum of 2 mL of contrast was injected. A second group of 70 rats underwent sham laparotomy with exposure of the IVC but no branch ligation or clip placement. Forty-eight hours later, they underwent a second sham operation followed by pulmonary angiography as described previously.

Animals were killed at 3 hours and then at 1, 2, 4, 6, 8, and 14 days after the PE. Ten animals were killed for each group at each time point. Half of these animals (n = 5 per group per time point) had the affected portion of the PA with some surrounding lung tissue dissected free, the

Day 4	*Day 6*	*Day 8*	*Day 14*	*ANOVA*
45.3 ± 15.8*†	11.5 ± 4.1*	1 ± 0.6	1.5 ± 1.5	$P < .01$
34.5 ± 10.4*†	9.0 ± 3.9	0	1.2 ± 1.2	$P < .01$
10.8 ± 6.8	2.0 ± 1.7	1.0 ± 0.6	0	$P < .01$
0	0.5 ± 0.5	0	0	NS
3.0 ± 2.0	1.7 ± 0.9	0	NS	
2.0 ± 1.7	1.0 ± 0.4	0	NS	
0.8 ± 0.3	0	0	NS	
0.25 ± 0.25	0.75 ± 0.5	0	NS	
0.6 ± 0.6	0	0.25 ± 0.25	NS	
0.2 ± 0.2	0	0.25 ± 0.25	NS	
0	0	0	NS	
0.4 ± 0.4	0	0	NS	

thrombus removed, and the tissue washed three times in 5 mL of phosphate buffered saline solution, snap frozen in liquid nitrogen, and stored at −70° C for future protein analysis. Sections of lung parenchyma distal to the site of PE were processed in a similar fashion. Sections of PA and distal lung parenchyma were taken from the sham animals and from the contralateral lung in the experimental group. The other half of these animals (n = 5 per group per time point) underwent PA and tracheal perfusion fixation with 10% buffered formalin phosphate (Fisher Scientific, Pittsburgh, Pa). The affected, contralateral, and sham PA and lung were removed and placed in 10% buffered formalin for 24 hours, followed by reagent alcohol 70% volume/volume (Labchem Inc, Pittsburgh, Pa) for subsequent permanent section processing. Ten animals were killed without any intervention for baseline controls, five for protein analysis and five for histologic analysis.

Oxygen saturation analysis. For assessment of the physiologic response to the induced PE, arterial oxygen saturation was measured. Before PE, immediately after PE, and before death, sedated rats breathing room air underwent dorsal tail arterial puncture. O_2 saturations then were measured on an OSM3 Hemoximeter (Radiometer Medical A/S, Denmark).

Cytokine and chemokine analysis and collagen assay. Specific cytokines and chemokines for study were chosen on the basis of their documented importance in vein wall inflammation after DVT formation.[7,9,10-12,17,18] The PA tissue and distal lung parenchyma were thawed, placed in complete lysis buffer (Boehringer Mannheim, Indianapolis, Ind), homogenized for 45 seconds with a hand-held homogenizer (TH, Omni International, Warrenton, Va), and sonicated for 30 seconds (Micro Ultrasonic Cell Disrupter, Kontes, Vineland, NJ). The homogenate was centrifuged at 10,000 *g* for 5 minutes, and the supernatant and sediment fractions separated. Measured levels of inflammatory mediators were normalized to total protein in the sample. Total protein quantitation was determined with a bicinchoninic acid protein assay (Pierce, Rockford, Ill) with serial dilutions of bovine serum albumin (Sigma Chemical, St Louis, Mo) as standards. Analyses of inflammatory mediators, tissue necrosis factor-α (TNF-α), interleukin-10, monocyte chemoattractant protein–1 (MCP-1), and tissue growth factor–β (TGF-β) were performed with commercially available, rat-specific, enzyme-linked immunosorbent assays (ELISA; Endogen, Woburn, Mass) with sensitivities of more than 10 pg/mL, more than 3 pg/mL, and more than 5 pg/mL, respectively. A standard colorimetric kit (Cayman Chemical, Ann Arbor, Mich) was used to measure total nitric oxide. P-selectin, E-selectin, and keratinocyte-derived chemokine (KC/GRO) were quantified in the tissue homogenate with a direct ELISA method as previously described.[17] The sensitivity was 0.5 ng/mL or more, and negligible background was found when testing the secondary antibody against the adsorbed antigens. Hydroxyproline levels in the lung parenchyma were determined with an acid hydrolysis colorimetric assay as previously described.[12] Hydroxyproline standards ranged from 0 to 100 μg/mL.

Histopathology analysis and immunohistochemical staining. Leukocyte extravasation into the PA wall was assessed as previously described.[7,9,17] Leukocyte morphometric analysis was performed in a blinded fashion on hematoxylin and eosin stained sections. Each rat had three sections reviewed, and the section with the most complete circumference was chosen. Five high-powered fields (×100) radially around the PA were assessed for neutrophils, monocytes, and lymphocytes on the basis of standard morphologic criteria, including nuclear size, cytoplasmic content, and cell size. Analysis began at the thrombus-vein wall interface and extended the width of one high-power field or up to, but not including, the lung parenchyma,

whichever was smaller. On samples with no thrombus, analysis began at the endothelial cell layer of the PA. In addition, morphometric analysis was performed on the lung parenchyma distal to the affected PA. Again, five high-powered fields in the distal segment underwent counts for neutrophils, monocytes, and lymphocytes as described previously. Morphometrics were confirmed by comparison with neutrophils and macrophages stained with rat anti-polymorphonuclears (PMNs) and ED-1 antibody, respectively, as previously described.[9]

Brillmeyer's trichrome stain was applied to paraffin embedded tissue to assess collagen distribution. After the sections were deparaffinized, they were placed in hematoxylin for 40 seconds, followed by 0.2% (weight/volume) acid fuchsin for 1 minute. This was followed by a 3-hour incubation at room temperature in 0.5% anilin blue, 2% Orange G, and 1% phosphomolybdic acid and a subsequent dehydration in 100% ethanol. All reagents were from Sigma Chemical.

Intimal hyperplasia was estimated with measurements made, in a blinded fashion, from the luminal surface to the internal and external elastic laminae at three separate, randomly selected locations along each PA and then averaged.[19] Digitized images were analyzed with Adobe Photoshop 5.0 (Adobe Systems Incorporated, San Jose, Calif).

Statistical evaluation and animal use. Statistical evaluation included mean ± standard error of the mean, analysis of variance, Bonferroni post hoc analysis, and unpaired Student *t* tests. All animals used in this study were housed and cared for in the University of Michigan Unit for Laboratory Animal Medicine under the direction of a veterinarian according to the *Principles of Laboratory Animal Care* (National Society for Medical Research) and *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 86-23, revised 1985).

RESULTS

Animal model.[20] Induction of PE was attempted in 87 animals, with 70 animals (80.4%) obtaining PE visible on pulmonary angiogram. Of the animals that did not show a PE, five died at surgery, one did not have an IVC thrombus develop, and 11 had no PEs visible on angiography. Of the 1 animals with no angiographically visible PE, six had no thrombus found at necropsy and five had a large thrombus retained in the right ventricle of the heart. PE distribution with angiography was: 72% to the right caudal lobar PA, 8.5% to the right cranial lobar PA, 2.9% to the right medial lobar PA, 7% to the main right PA, and 8.5% to a branch of the left PA.

Arterial O_2 saturations were similar between experimental and sham animals at baseline (96.5% ± 0.4% versus 97.3% ± 0.3%, respectively). After PE, experimental animals had lower O_2 saturations compared with sham animals (87.7% ± 1.5% versus 96.4% ± 0.4%, respectively; $P < .01$). This reduction in O_2 saturation resolved by 1 day after PE.

Thrombus within the PA was visible at death at the early time points. At all time points with thrombus present, thrombus was not adhered to the PA wall. Mean thrombus resolution after PE (determined at death by the absence of thrombus in the PA) was 40% at 2 days, 90% at 4 days, and 100% at 6 days. In one instance, thrombus not visualized with angiography was found within the PA of the contralateral lung at the 3-hour time point. No PA thrombus was found in any of the sham animals.

Histologic evaluation and morphometric analysis of pulmonary artery after pulmonary embolism. At baseline, almost no inflammatory cells were present in the PA wall (Table I). Similarly, at each time point, few inflammatory cells were within the PA wall in sham animals (Fig 1, *A*), and no differences were seen from baseline in histologic appearance or morphometric analysis. At 3 hours after PE, evidence was found of inflammatory cell infiltration within the outer margin of the PA wall. Compared with sham animals, there was a 38-fold increase in leukocytes ($P < .01$), with a differential of 62% ± 18% neutrophils ($P < .01$) and 38% ± 19% macrophages. At scattered points along the embolus, neutrophils lined the outer edge. By 1 and 2 days after PE, there was increased neutrophil and macrophage infiltration into the PA wall (Fig 1 , *B*) and evidence of PMNs within the PA wall vasovasorum. At 2 days after PE, total leukocyte count increased 320-fold compared with sham animals ($P < .01$), with 72% ± 22% neutrophils ($P < .01$) and 28% ± 11% macrophages. At 4 days after PE, most thrombi had lysed and a concurrent decrease in inflammatory cell infiltrate was observed. Total leukocyte count dropped to a 45-fold ($P < .01$) increase over sham animals, with 77% ± 21% neutrophils ($P < .01$). At 14 days after PE, the PA wall appeared similar to baseline with no significant difference in leukocyte count. On examination of the contralateral PA at all time points, no histologic or morphometric differences were seen compared with sham animals or with baseline.

Evaluation of the PA intima and media revealed a change over time as well. Starting at 4 days after PE, there was an increase in intimal thickness. This was associated with increased cellularity of the intimal layer as compared with sham animals (Fig 2, *A* and *B*). The intimal hypercellularity persisted through 14 days after PE. Although the identity of these cells is not clear, they do not stain with PMN or macrophage-specific markers and likely represent proliferating smooth muscle cells. In addition, at both time points, the medial smooth muscle cells developed more rounded nuclei and cellular structure and appeared to have more evidence of mitoses. Trichrome staining allowed PA intimal and medial thickness to be quantified. Two-day, 4-day, 6-day, and 14-day time points were evaluated. Compared with sham animals, there was a significant increase in the intima to media ratio apparent at 4 days (0.23 versus 0.12; $P < .01$), 6 days (0.23 versus 0.11; $P < .05$), and 14 days (0.27 versus 0.14; $P < .05$; Fig 2 , *C*).

Histologic evaluation and morphometric analysis of lung parenchyma after pulmonary embolism. Morphometric analysis was performed on distal lung segments at baseline and after PE at time points 3 hours and 1, 2, 4, and 14 days. At baseline, there were predominantly macrophages within the lung parenchyma, with few neutrophils

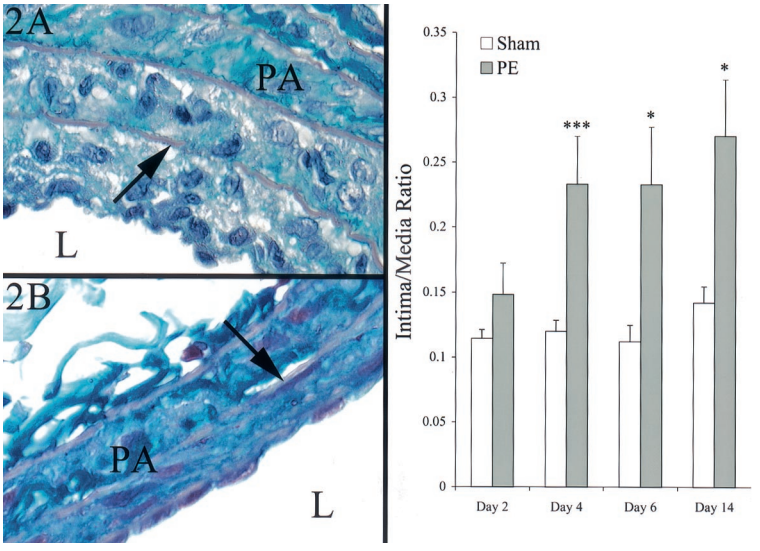

Fig 2. Photomicrographs show: **A,** intimal hyperplasia in PA wall 4 days after PE; and **B,** no intimal hyperplasia in sham animals at 4 days after PE. Trichrome stain; original magnification, 100×. **C,** Graphic representation of intima to media ratio from experimental and sham animals.
*$P < .05$.
***$P < .01$.
L, Lumen; *solid arrow,* internal elastic lamina.

Table II. Morphometric analysis (cells per five high-power fields) of inflammatory cells in distal lung parenchyma indexed to time from PE

	Baseline	*3 Hours*	*Day 1*	*Day 2*	*Day 4*	*Day 14*	*ANOVA*
PE							
Affected lung (n = 6)							
Total leukocytes	50.6 ± 3.8	97.0 ± 8.4†	65.0 ± 8.3	76.6 ± 2.9*	75.4 ± 5.0*	66.5 ± 6.8	$P < .01$
Neutrophils	7.2 ± 1.3	11.2 ± 1.5	5.6 ± 1.6	8.6 ± 1.4	10.6 ± 1.5	11.2 ± 1.6	NS
Macrophages	43.4 ± 2.6	85.8 ± 9.2*†	59.4 ± 8.0*	68.0 ± 3.6*	64.8 ± 5.3*	55.4 ± 5.3	$P < .01$
Contralateral lung (n = 6)							
Total leukocytes		79.2 ± 7.5	81.4 ± 13.3*	84.0 ± 5.1*	92.2 ± 3.4*†	71.6 ± 4.6†	$P < .01$
Neutrophils		27.8 ± 9.5	8.0 ± 2.8	14.6 ± 4.0	8.6 ± 1.5	16.0 ± 2.3	NS
Macrophages		51.4 ± 5.1*	73.4 ± 11.2*†	69.4 ± 3.3*†	83.6 ± 3.8*†	55.6 ± 2.7	$P < .01$
Sham lung (n = 6)							
Total leukocytes		70.2 ± 6.0	37.0 ± 5.6	49.4 ± 4.3	53.6 ± 5.7	54.6 ± 4.1	$P < .01$
Neutrophils		24.0 ± 4.7	13.0 ± 1.6†	17.2 ± 2.6	12.8 ± 1	12.6 ± 3.2	$P < .01$
Macrophages		46.2 ± 3.9	24.0 ± 4.7†	32.2 ± 2.5	40.8 ± 5.5	42.0 ± 2.3	$P < .01$

*$P < .01$ compared with sham PA.
†$P < .01$ compared with baseline.
ANOVA, Analysis of variance; *NS*, not significant.

(Table II). After PE, there was a significant increase in the number of macrophages present compared with sham animals (Table II). This difference was first present at 3 hours and persisted through 4 days after PE. Unlike the PA wall, the number of neutrophils did not significantly increase at any of the time points. A similar inflammatory response was present in the distal lung segment from the contralateral lung, with significant elevations in total leukocytes and macrophages at 2 and 4 days ($P < .01$; Table II).

Cytokine and chemokine levels within the pulmonary arterial wall after pulmonary embolism.[20] Cytokines and chemokines in the PA wall, after PE, were quantified with ELISA. KC/GRO, interleukin-10, TNF-α, and nitric oxide levels did not differ significantly among the groups, and P-selectin and E-selectin levels were not detectable in the PA wall. KC/GRO and interleukin-10 levels were significantly elevated in affected PAs, contralateral PAs, and sham PAs compared with baseline controls. Only MCP-1 levels from the affected PA wall were elevated compared with the sham PAs (Fig 3), and this elevation occurred at 2 days after PE. MCP-1 levels also were significantly increased from baseline (115 ± 30 pg/mg protein) at day 1 after PE (285 ± 40 pg/mg protein; $P < .01$) in the affected PA from the experimental group (Fig 3) and the

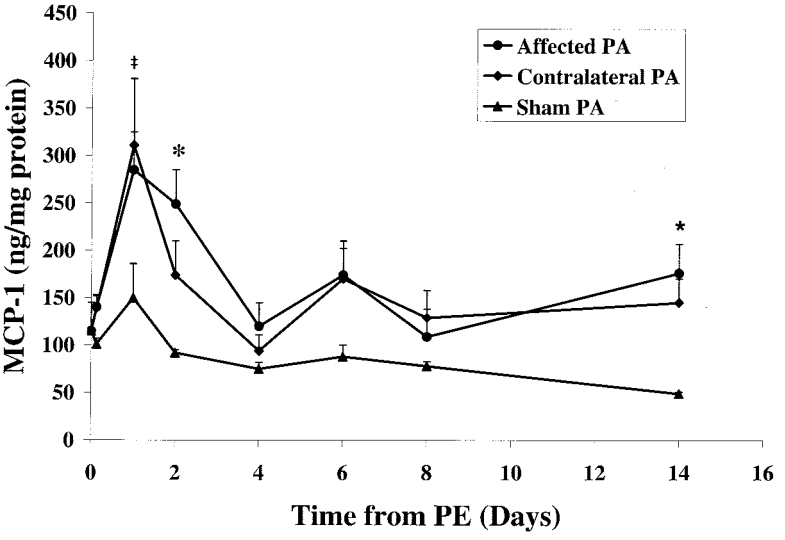

Fig 3. Graphic representation of MCP-1 levels (pg/mg protein) over time from affected and contralateral PA wall in experimental (PE) animals and from PA wall of sham animals.
*$P < .01$ compared with sham.
‡$P < .01$ compared with baseline.

contralateral PA (311 ± 70 pg/mg protein; $P < .01$). At no time did MCP-1 levels from sham PAs increase over baseline.

Collagen and inflammatory chemokine and cytokine levels within the lung parenchyma after pulmonary embolism. MCP-1, hydroxyproline, and TGF-β levels were measured in lung tissue at baseline and at 3 hours and 1, 2, 4, and 14 days after PE. There were no significant differences in the MCP-1 levels among affected lungs, contralateral lungs, and sham lungs. There was a trend, however, toward elevated MCP-1 levels at 1 and 2 days after PE, mirroring the results seen in the PA wall ($P = .12$ and $P = .4$). Hydroxyproline was not significantly different among the groups nor compared with baseline. TGF-β was suppressed in both lungs of experimental animals compared with shams at 3 hours after PE (152 ± 18 pg/mg protein and 128 ± 38 pg/mg protein versus 383 ± 87 pg/mg protein; $P < .01$) but had no difference at any other time point.

DISCUSSION

For examination of the inflammatory effect of PE on the PA wall and parenchyma, a reproducible model of PE was developed in a small animal. The strengths of this model are that the PE more closely resembles the clinical disease, with an in situ thromboembolus traveling into the pulmonary circulation. This differs from most previously reported studies in which exogenous thrombus or other material are injected into the venous or PA circulation.[21,22] This model is reproducible with an 80.4% success rate, which is similar to models in which exogenous clot was introduced into the venous circulation.[22] Other benefits of this model are that it allows for accurate radiographic localization of the PE within specific PA segments. Finally, the PE's physiologic effect was marked by transient hypoxia with subsequent resolution, not unlike the situation in humans. No other small animal models have documented this physiologic response. There was, however, variability in the timing of thrombus resolution, which may reflect variability in the size of the thrombus. No correlation could be made between the time of thrombus resolution and corresponding cytokine and chemokine levels or degree of inflammatory cell infiltration. In addition, no evidence was seen of pulmonary infarction. This is likely the result of sufficient collateral blood supply to the affected lung tissue provided by other PA branches or bronchial arteries.

Mechanisms that may account for the physiologic manifestations of PE also were suggested by this study. PE is associated with a leukocyte influx, not only in the PA wall but also the parenchyma. This influx of leukocytes occurs early after PE (1 day). In the PA wall, the inflammatory cell population is predominately neutrophils but with a concomitant rise in macrophages. In the parenchyma, the inflammatory cell response is predominantly that of macrophages. Temporally, this rise in PA wall neutrophils correlates with the cellular inflammatory response seen in the DVT model.[7,8] The early rise in the macrophage cell line, however, is different. In the animal models of DVT, the macrophages infiltrate the vein wall, becoming the predominate leukocyte at approximately day 3 after thrombus formation.[7] The timing of the influx of macrophages within the PA wall does correlate with that in the vein wall after DVT if the frame of reference is from the time of clot formation. This suggests that the thromboembolus may direct PA wall and parenchymal cellular events and not primarily the PA.

Of all of the chemokines and cytokines assessed after PE, only MCP-1 was elevated within the PA wall in experimental animals compared with sham animals. This elevation peaked at 2 days after PE and preceded the peak of macrophage influx. This differs from the response reported after development of thrombus in the IVC. Within the IVC wall, epithelial neutrophil activating protein–78, TNF-α, interleukin-6, MCP-1, macrophage inflammatory protein–1, and P-selectins all rise.[7,18] Interestingly, P-selectin was not detected in the PA wall after PE. This is distinctly different from DVT genesis in which P-selectin is important in mediating leukocyte interaction with endothelial cells, other leukocytes, and platelets.[23,24] In DVT models, blockade of P-selectin has been found to decrease associated perithrombotic inflammation and thrombosis propagation but not leukocyte vein wall influx.[9-11] Its inhibition or deletion, however, does not completely prevent inflammatory cell infiltration into the vessel wall, which likely relies on several different cellular adhesion molecules. Furthermore, the inflammatory cells in the PA wall after PE may originate from the lung parenchyma and rely on a completely different mechanism of infiltration. The lack of selectin elevation in the PA wall after PE suggests that the selectins may have little role in promoting inflammatory cell extravasation but may be more important in the thrombogenesis.

MCP-1 and monocyte/macrophages likely play an important role in embolism resolution. MCP-1 is a chemoattractant and activator of monocytes and can be produced by a variety of cell types within a resolving thrombus.[25-27] Monocytes, the precursors of tissue macrophages, produce tissue plasminogen activator and urokinase.[28,29] Monocytes, however, also produce inhibitors of fibrinolysis, whose procoagulant activity may be mediated by P-selectin, which is not elevated in this model.[30-32] There is an accumulation of macrophages that occurs within both the PA wall and the parenchyma after PE and corresponds to the MCP-1 PA wall kinetics. This rise is temporally associated with thrombus resolution. MCP-1 may shift the macrophage function towards fibrinolysis. Humphries and colleagues[33] have shown that after thrombus formation in rats, MCP-1 levels increase in blood, thrombi, and adjacent vessel wall. In addition, injection of MCP-1 into newly formed thrombus increased organization and resolution of the thromubs.[33] In this model, the mechanism of action may be through recruitment of macrophages to the site of embolism or through the differentiation of these cells into macrophages secreting more fibrinolytic proteins than inhibitors to allow PE resolution.

Other inflammatory mediators likely play a role in the PA wall inflammatory response, but none were detected in this model. The elevation of KC/GRO and interleukin-10 in both sham and experimental animals compared with baseline controls suggests that there was a systemic inflammatory response that is likely attributable to the operations and the angiography. Other inflammatory mediators may have been overshadowed because of this response. The importance of MCP-1 in this model is further strengthened, however, because it was not elevated in sham animals compared with baseline controls and thus its elevation in experimental animals can be attributed to the PE.

MCP-1 and the macrophage cell line also may play an important role in the development of intimal hyperplasia observed after PE. The PA intimal region becomes thickened and hypercellular in this PE model shortly after the peak elevations of MCP-1 and macrophages. Experimental evidence suggests that MCP-1 may play an important role in the development of intimal hyperplasia. In models with vein grafts, the development of intimal hyperplasia is associated with the presence of monocytes and macrophages and the upregulation of MCP-1 gene expression.[34] Depletion of macrophages in a rat model of vein graft intimal hyperplasia leads to suppression of intimal hyperplasia with resumption of the process as macrophages return.[35] Triazolopyrimidine has been shown to attenuate intimal hyperplasia in a balloon injury model.[36] This occurs by reducing MCP-1 expression and inhibiting arterial wall macrophage accumulation. Finally, after carotid artery injury in rats, intimal hyperplasia is inhibited by treatment with an anti–MCP-1 antibody.[37] Treatment with recombinant MCP-1, however, did not promote intimal hyperplasia. Further work is necessary to evaluate the development of intimal hyperplasia in the PA wall after a PE and to further define the role of MCP-1 and macrophages in this process and the overall process of pulmonary inflammation.

No evidence was found of significant pulmonary parenchymal fibrosis after one major PE. This is supported by the lack of qualitative differences in the collagen staining among the groups and by no differences in the levels of parenchymal hydroxyproline. TGF-β has been shown to be a regulator of pulmonary fibrosis after lung injury.[38,39] After one PE, despite a local and regional inflammatory response, TGF-β levels were initially suppressed before returning to levels equivalent to sham animals. Although TGF-β has been important in mediating acute lung injury, its role in mediating lung injury after PE is less clear.[40] Thus, in this animal model of single large PE, parenchymal fibrosis does not occur, but persistence of PA fibrosis does (at least through 14 days). Given the physiologic relevance of this model, further experiments will determine how PA injury can be attenuated and how best to apply these therapies clinically.

REFERENCES

1. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ III. Trends in the incidence of deep vein thrombosis and pulmonary embolism. Arch Intern Med 1998;158:585-93.
2. Proctor MC, Greenfield LJ. Pulmonary embolism: diagnosis, incidence and implications. Cardiovasc Surg 1997;5:77-81.
3. Menendez R, Nauffal D, Cremades MJ. Prognostic factors in restoration of pulmonary flow after submassive pulmonary embolism: a multiple regression analysis. Eur Respir J 1998;11:560-4.
4. Morrell MT, Dunnill MS. Fibrous band in conducting pulmonary arteries. J Clin Pathol 1967;20:139-44.
5. Moser KM, Auger WR, Fedullo PF. Chronic major-vessel thromboembolic pulmonary hypertension. Circulation 1990;81:1735-43.
6. Wagenvoort CA. Pathology of pulmonary thromboembolism. Chest 1995;107:10S-7S.

7. Wakefield TW, Strieter RM, Wilke CA, Kadell AM, Wrobleski SK, Burdick MD, et al. Venous thrombosis-associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. Arterioscler Thromb Vasc Biol 1995;15:258-68.
8. Downing LJ, Strieter RM, Kadell AM, Wilke CA, Brown SL, Wrobleski SK, et al. Neutrophils are the initial cell type identified in deep venous thrombosis induced vein wall inflammation. ASAIO J 1996;42:M677-82.
9. Wakefield TW, Strieter RM, Downing LJ, Kadell AM, Wilke CA, Burdick MD, et al. P-selectin and TNF inhibition reduce venous thrombosis inflammation. J Surg Res 1996;64:26-31.
10. Downing LJ, Wakefield TW, Strieter RM, Prince MR, Londy FJ, Fowlkes JB, et al. Anti-P-selectin antibody decreases inflammation and thrombus formation in venous thrombosis. J Vasc Surg 1997;25:816-28.
11. Eppihimer MJ, Schaub RG. P-selectin-dependent inhibition of thrombosis during venous stasis. Arterioscler Thromb Vasc Biol 2000;20: 2483-8.
12. Henke PK, Wakefield TW, Kadell AM, Linn MJ, Varma MR, Sarkar M, et al. Interleukin-8 administration enhances venous thrombosis resolution in a rat model. J Surg Res 2001;99:84-91.
13. Lang IM, Moser KM, Schleef RR. Elevated expression of urokinase-like plasminogen activator and plasminogen activator inhibitor type 1 during the vascular remodeling associated with pulmonary thromboembolism. Arterioscler Thromb Vasc Biol 1998;18:808-15.
14. Reyers I, Mussoni L, Donati MB, de Gaetano G. Failure of aspirin at different doses to modify experimental thrombosis in rats. Thromb Res 1980;18:669-74.
15. Millet J, Thevaniaux J, Pascal M. A new experimental model of venous thrombosis in rats involving partial stasis and slight endothelial alterations. Thromb Res 1987;45:123-33.
16. Monreal M, Silveira P, Monreal L, Monasterio J, Angles AM, Lafoz E, et al. Comparative study on the anti-thrombotic efficacy of four low-molecular-weight heparins in three different models of experimental venous thrombosis. Haemostasis 1991;21:91-7.
17. Henke PK, DeBrunye LA, Strieter RM, Bromberg JS, Prince M, Kadell AM, et al. Viral IL-10 gene transfer decreases inflammation and cell adhesion molecule expression in a rat model of venous thrombosis. J Immunol 2000;164:2131-41.
18. Myers D, Farris D, Hawley A, Wrobleski S, Chapman A, Stoolman L, et al. Selectins and interleukin-10 influence acute thrombus formation in a mouse model of venous thrombosis. J Surg Res. In press 2002.
19. Sarac TP, Riggs PN, Williams JP, Feins RH, Baggs R, Rubin P, et al. The effects of low-dose radiation on neointimal hyperplasia. J Vasc Surg 1995;22:17-24.
20. Eagleton MJ, Henke PK, Luke CE, Wakefield TW, Greenfield LJ. Pulmonary embolism is associated with increased pulmonary arterial wall MCP-1 levels. Surg Forum 2001;LII:342-3.
21. Clozel JP, Holvoet P, Tschopp T. Experimental pulmonary embolus in the rat: a new in vivo model to test thrombolytic drugs. J Cardiovasc Pharmacol 1988;12:520-5.
22. Stassen JM, Lijnen HR, Kieckens L, Collen D. Small animal thrombosis models for the evaluation of thrombolytic agents. Circulation 1991;83: IV65-72.
23. Walchek B, Moore KL, McEver RP, Kishimoto TK. Neutrophil-neutrophil interactions under hemodynamic shear stress involve L-selectin and PSGL-1. A mechanism that amplifies intitial leukocyte leukocyte accumulation of P-selectin in vitro. J Clin Invest 1996;98:1081-7.
24. Konstantopoulos K, Neelamegham S, Burns AR, Hentzen E, Konsas GS, Snapp KR, et al. Venous levels of shear support neutrophil-platelet adhesion and neutrophil aggregation in blood via P-selectin and β_2-integrin. Circulation 1998;98:873-82.
25. Ajuebor MN, Flower RJ, Hannon R, Christir M, Bowers K, Verity A, et al. Endogenous monocyte chemoattractant protein-1 recruits monocytes in the zymosan perinotitis model. J Leukoc Biol 1998;63:108-16.
26. Conti P, Pang X, Boucher W, Letourneau R, Reale M, Barbacane RC, et al. Monocyte chemotactic protein-1 is a proinflmamatory cytokine in rat skin injection sites and chemoattracts basophilic granuylar cell. Int Immunol 1997;9:1563-70.
27. Colotta F, Sciacca FL, Sironi M, Luini W, Raviet MJ, Mantovbani A. Expression of monocyte chemotactic protein-1 by monocytes and endothelial cells exposed to thrombin. Am J Pathol 1994;144:975-85.
28. Lundgren CH, Sawa H, Soble BE, Fujii S. Modulation of expression of monocyte/macrophage plasminogen activator activity and its implication for attenuation of vasculopathy. Circulation 1994;90:1927-34.
29. Kung SKP, Lau HKF. Modulation of the plasminogen activation system in murine macrophages. Biochim Biophys Acta 1993;1176:113-22.
30. Castellote JC, Grau E, Linde MA, Pujol-Marx N, Rutllant ML. Detection of both type-1 and type-2 plasminogen activator inhibitors in human monocytes. Thromb Haemost 1990;63:67-71.
31. Edgington TS, Mackman N, Brand K, Ruf W. The structural biology of expression and function of tissue factor. Thromb Haemost 1991;66:67-79.
32. Burnand KG, Gaffney PJ, McGuinness CL, Hjmphries J, Quarmby JW, Smith A. The role of the monocyte in the generation and dissolution of arterial and venous thrombi. Cardiovasc Surg 1998;6:119-25.
33. Humphries J, McGuinness CL, Smith A, Waltham M, Poston R, Burnand K. Monocyte chemotactic protein-1 (MCP-1) accelerates the organization and resolution of venous thrombi. J Vasc Surg 1999;30: 894-900.
34. Stark VK, Hoch JR, Warner TF, Hullett DA. Monocyte chemotactic protein-1 expression is associated with the development of vein graft intimal hyperplasia. Arterioscler Thromb Vasc Biol 1997;17:1614-21.
35. Hoch JR, Stark VK, van Rooijen N, Kim JL, Nutt MP, Warner TF. Macrophage depletion alter vein graft intimal hyperplasia. Surgery 1999;126:428-37.
36. Poon M, Cohen J, Siddiqui Z, Fallon JT, Taubman MB. Trapidil inhibits monocyte chemoattractant protein-1 and macrophage accumulation after balloon arterial injury in rabbits. Lab Invest 1999;79:1369-75.
37. Furukawa Y, Matsumori A, Ohashi N, Shioi T, Ono K, Harada A, et al. Anti-monocyte chemoattractant protein-1/monocyte chemotactic and activating factor antibody inhibits neointimal hyperplasia in injured rat carotid arteries. Circ Res 1999;84:306-14.
38. Phan S, Gharaee-Kermani M, Wolber F, Ryan US. Stimulation of rat endothelial cell tranforming growth factor-beta production by bleomycin. J Clin Invest 1991;87:148-54.
39. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor β on bleomycin-induced accumulation of lung collagen in mice. Thorax 1993;38:959-66.
40. Pittet JF, Griffiths MJD, Geiser T, Kaminski N, Dalton SL, Huan X, et al. TGF-β is a critical mediator of acute lung injury. J Clin Invest 2001;107:1537-44.

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